Effects of long-term treatment with fluoxetine and venlafaxine on rat isolated vas deferens

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Summary
1. Antidepressant therapy is considered as one of the factors leading to male infertility.
2. In this study, the effects of long-term treatment with fluoxetine or venlafaxine were investigated on electrical field stimulation (EFS, 1–64 Hz), noradrenaline (10⁻⁸ to 10⁻⁴ M), serotonin (10⁻⁸ to 10⁻⁴ M), adenosine 5′-triphosphate [ATP (10⁻⁸ to 10⁻⁴ M)] and 80 mM KCl-induced contractile responses in the epididymal and prostatic portions of rat isolated vas deferens strips.
3. Serotonin-induced contractile responses were significantly increased in the epididymal portion of the vas deferens obtained from the fluoxetine-treatment group, whereas in the prostatic portion there was no change. However, venlafaxine treatment had no effect on serotonin responses in the either portion of the vas deferens. Both fluoxetine and venlafaxine treatment significantly inhibited ATP-evoked contractions of the prostatic and epididymal portions of the rat vas deferens, but had no effect on EFS, noradrenaline- and KCl-evoked contractions of the vas deferentia in both portions.
4. In conclusion, these results suggest that chronic treatment with fluoxetine and venlafaxine affects vas deferens motility. Purinoceptors may, at least in part, responsible for the impaired motility in chronic treatment of venlafaxine and fluoxetine.

Keywords: fluoxetine, venlafaxine, noradrenaline, serotonin, ATP, electrical field stimulation, vas deferens, rat

Introduction
There is increasing awareness of sexual dysfunction as a potential side effect of antidepressant therapy (Walsh et al., 1992; Montgomery et al., 2002). Several studies have indicated that such problems are common (Rothschild, 2000; Taylor et al., 2005). Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), and venlafaxine, a serotonin and noradrenaline reuptake inhibitor (SNRI), are used for the treatment of depression but also associated with a high incidence of sexual dysfunction such as delayed or absent ejaculation (Montgomery et al., 2002; Dording et al., 2002; Masand & Gupta, 2002; Waldinger et al., 1998a; Rosen et al., 1999). These sexual side effects can considerably affect a person’s lifestyle, and where this results in reduced compliance with medication, lead to less effective treatment of the primary psychiatric disorder. The mechanisms by which antidepressants cause sexual dysfunction involve complex multi-system interactions, which are not entirely understood, and psychological factors must also be considered.

It is well known that ejaculation occurs in response to rhythmic contractions of male secondary sex organs including vas deferens (Steers, 1994; Vale, 1999; Ralph & Wylie, 2005). Reduced motility of the vas deferens was shown to contribute to male infertility (Chinoy & Chinoy, 1981; Mulrayn et al., 2000). Pharmacology research into human ejaculatory disorders is limited to clinical studies with registered drugs affecting the ejaculation process; therefore animal research seems a prerequisite. With this background the aim of this work was to investigate the effects of fluoxetine and venlafaxine on electrical field stimulation (EFS), noradrenaline, adenosine 5′-triphosphate (ATP), serotonin and KCl-induced contractions of the vas deferens in order to evaluate the effect of fluoxetine and venlafaxine on the motility of the vas deferens.

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Materials and methods

Animals

Adult male Wistar rats weighing 250–300 g obtained from Experimental Medical Research Center (DETAB, Kocaeli University, Kocaeli, Turkey), were placed in a quiet, temperature- and humidity-controlled room (22 ± 3 °C and 62 ± 7%, respectively) in which a 12–12 h light-dark cycle was maintained (07:00–19:00 h light). The experiments reported in this study were carried out in accordance with the Regulation of Animal Research Ethics Committee in Turkey (6 July 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey).

Treatment and experimental schedule

Animals were divided into three groups: the fluoxetine group (n = 10) were treated by ip injection of fluoxetine (20 mg kg⁻¹ day⁻¹) and venlafaxine group (n = 10) were treated by ip injection of venlafaxine (20 mg kg⁻¹ day⁻¹) during 14 days. Rats receiving only the vehicle ip (0.9% saline) during 14 days served as control group (n = 10). After 14 days of treatment, all animals were killed under ether anaesthesia, the abdomen was opened and the vas deferens from each side removed. The vas deferens was divided into prostatic and epididymal halves of 1–2 cm in length described by Ventura (1998). The epididymal and prostatic portion were placed in a 20 ml organ bath containing Krebs Henseleit solution containing (mM): 113 NaCl, 4.8 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 11.7 glucose. The solution was gassed with 95% O₂ and 5% CO₂ during the study. The temperature was maintained at 37 °C by a thermoregulated water circuit. Each strip was connected to a force transducer (FDT 10 A Commat Iletisim, Ankara, Turkey) for the measurement of isometric force, which was continuously recorded on a computer via a four-channel transducer data acquisition system (TDA 94, Commat Iletisim) using software (Polywin 95 ver 1.0, Commat Iletisim) that also had the capacity to analyse the data.

Results

Electrical field stimulation was provided by a stimulator (ST 95PT, Commat Iletisim) and applied via two platinum wire electrodes set vertically in the organ bath either side of the suspended tissue. Square-wave pulses of 40 V, 0.5 ms duration in 10 s trains with varying frequency (1–64 Hz) were applied at 5-min intervals. The strips were allowed to return to baseline tension between the tests at each frequency.

Analysis of data

Results are expressed as the mean ± SEM of different experiments. Contractile responses to noradrenaline, ATP, serotonin and EFS were calculated as percentage of the maximal contraction caused by KCl (80 mM). To evaluate the effects of agonists, maximum responses (Eₘₐₓ) and pD₂ values (apparent agonist affinity constants; −logEC₅₀) were calculated. Agonist E₅₀ values were calculated from each agonist concentration–response.

Statistical comparison between the groups was performed using ANOVA supported by Dunnett’s post hoc test. Results were considered to be significantly different at a P-value of <0.05.

Drugs

The following drugs were used: noradrenaline bitartrate (Sigma Chemical, St. Louis, MO, USA), serotonin creatinine sulphate (Sigma Chemical), ATP (Sigma), fluoxetine hydrochloride (Deva, Istanbul, Turkey) and venlafaxine hydrochloride (Wyeth, Istanbul, Turkey). All drugs were dissolved in 0.9% saline and were freshly prepared on the day of the experiments.

Electrical field stimulation delivered at frequencies from 1 to 64 Hz (Tables 1 & 2) to both portions of the rat vas deferens elicited reproducible, frequency-dependent, contractile activity which was not changed by either fluoxetine or venlafaxine treatment.

Serotonin (10⁻⁸ to 10⁻⁴ M) elicited concentration-dependent contractions in the both portions of vas deferens isolated from rats in all groups (Fig. 1, Tables 1 & 2). Serotonin-induced contractions were significantly increased in epididymal portion of the rat vas deferens obtained from fluoxetine group compared with control group (P < 0.05; Fig. 1), while it did not change contractile responses obtained from the prostatic portion (Tables 1 & 2). The Eₘₐₓ value for serotonin was significantly higher in the epididymal portions of the rat vas deferens obtained from fluoxetine-treated groups than in the control group (P < 0.05; Table 1). Venlafaxine treatment had no effect on the serotonin-induced contractile

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After mounting, each strip was allowed to equilibrate Krebs Henseleit solution. At the end of the equilibration, strips KCl (80 mM). To evaluate the effects of agonists, maximum responses (Eₘₐₓ) and pD₂ values (apparent agonist affinity constants; −logEC₅₀) were calculated. Agonist E₅₀ values were calculated from each agonist concentration–response.

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Table 1  $E_{\text{max}}$ (% of 80 mM KCl) values for noradrenaline, ATP, serotonin and EFS and $E_{\text{max}}$ value (mg) for 80 mM KCl in vas deferens obtained from fluoxetine, venlafaxine treatment and control rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fluoxetine</th>
<th>Venlafaxine</th>
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<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$n$</td>
<td>$n$</td>
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<tr>
<td>Epididymal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>1885 ± 207</td>
<td>1667 ± 131</td>
<td>1447 ± 160</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>47 ± 4</td>
<td>55 ± 4</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>ATP</td>
<td>22 ± 5</td>
<td>5 ± 2*</td>
<td>6 ± 1*</td>
</tr>
<tr>
<td>Serotonin</td>
<td>24 ± 3</td>
<td>38 ± 3*</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>EFS</td>
<td>296 ± 33</td>
<td>273 ± 13</td>
<td>267 ± 26</td>
</tr>
<tr>
<td>Prostatic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>4347 ± 383</td>
<td>10 4971 ± 384</td>
<td>3704 ± 271</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>14 ± 1</td>
<td>18 ± 2</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>ATP</td>
<td>9 ± 1</td>
<td>3 ± 1*</td>
<td>6 ± 1*</td>
</tr>
<tr>
<td>Serotonin</td>
<td>9 ± 2</td>
<td>12 ± 3</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>EFS</td>
<td>103 ± 12</td>
<td>121 ± 12</td>
<td>123 ± 14</td>
</tr>
</tbody>
</table>

*P < 0.05, statistically different from the response of muscles from control rats.

EFS, electrical field stimulation.

Table 2  $pD_2$ values for noradrenaline, ATP, serotonin in vas deferens muscles obtained from fluoxetine, venlafaxine treatment and control rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
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<th>Venlafaxine</th>
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<tr>
<td>Epididymal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>5.5 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>ATP</td>
<td>5.4 ± 0.1</td>
<td>5.6 ± 0.3</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5.4 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Prostatic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>5.7 ± 0.4</td>
<td>5.3 ± 0.2</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>ATP</td>
<td>5.2 ± 0.1</td>
<td>5.4 ± 0.2</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5.9 ± 0.2</td>
<td>5.6 ± 0.2</td>
<td>5.6 ± 0.3</td>
</tr>
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</table>

Values are arithmetic means ± SE, $n$ = the number of rats.

Discussion

The results of our study show that serotonin-induced contractions are significantly increased in epididymal portions of vas deferens obtained from fluoxetine-treated rats but there were no significant differences in prostatic portions. However, ATP-induced contractions are significantly reduced in both portions obtained from fluoxetine and venlafaxine-treated animals. A previous study showed that the epididymal and prostatic portions of vas deferens are mainly innervated by the noradrenergic and purinergic system, respectively (Boselli et al., 1998). Therefore, the epididymal and prostatic portions had different contraction characteristics. For these reasons, we studied both portions of vas deferens separately.

Serotonin is believed to be an inhibitory transmitter in the control of sexual drive (Walsh et al., 1992; Yells et al., 1994). High central serotonin levels are associated with the inhibition of ejaculation (Zajecka et al., 1991). However, there is no evidence that serotonin is an important neurotransmitter in the vas deferens. We found similar results with a previous study which demonstrated an increased contractile response to serotonin in epididymal portions of vas deferens obtained from fluoxetine-treated rats compared with the control group (Busch et al., 1999). Also, a slight increase in serotonin responses were observed in prostatic portions of vas deferens obtained from fluoxetine-treated rats compared with control group although these differences did not attain statistical significance. Similarly, De Jong et al. (2005) demonstrated that chronic treatment with paroxetine, another SSRI, significantly inhibited and delayed ejaculation in rats. This is consistent with previous observations that administration of various SSRI such as fluoxetine, sertraline and paroxetine significantly increased ejaculation latency in patients with premature ejaculation and this is evidence that serotonin plays an important role in delaying ejaculation (Sae Chul & Kyung Keun, 1998; Waldinger et al., 1998a,b; Waldinger & Olivier, 2005). In contrast to the fluoxetine group, no change was found in serotonin responses in rats treated with venlafaxine compared to control rats.

Sympathetic transmission is mainly mediated through noradrenaline and ATP, which are co-transmitters present in sympathetic nerves in the vas deferens of rat, rabbit and guinea-pig (Sneddon & Machalay, 1992). It is well known that combined adrenergic and purinergic mechanisms are
necessary for contraction of the vas deferens. EFS evokes biphasic contractions in the rat vas deferens, the first phase is attributed to ATP acting at P2x receptors and the second phase to noradrenaline at α1-receptors (Sneddon et al., 1984; Ventura, 1998).

In this study, EFS-evoked contractile responses were not changed in either portion of the vas deferens obtained from fluoxetine- and venlafaxine-treated rats. Thus, it is unlikely that long-term fluoxetine and venlafaxine treatment affects vas deferens motility by reducing neurotransmitter release.

 Venlafaxine blocks the reuptake of both serotonin and noradrenaline. Although fluoxetine has been considered as a SSRI, it is known to inhibit noradrenaline uptake in brain (Stanford, 1996). Also it is reported that fluoxetine exerts a dual effect on noradrenaline-induced rat vas deferens contractions; it increases the response to low noradrenaline concentrations, as a consequence of a blockade of

**Figure 1** Electrical field stimulation (EFS; 40 V, 1–64 Hz, 10 s train)-induced contractions in the isolated epididymal segment of rat vas deferens smooth muscles.

**Figure 2** Serotonin concentration–responses curves in isolated epididymal segment of rat vas deferens smooth muscles. Each point is expressed as a percentage of the contraction induced by 80 mM KCl is given as the mean ± standard error of the mean (SEM). Number of rats in each group is shown in parentheses.
uptake (Sammet & Graefe, 1979) and decreases the maximal response to noradrenaline by inhibiting calcium fluxes (Busch et al., 2000). Previously it has been shown that $10^{-5}$ M fluoxetine enhanced the contractile response to noradrenaline in isolated rat vas deferens but no such effect was found at $10^{-7}$ or $10^{-6}$ M fluoxetine (Velasco et al., 1997). Also Busch et al. (1999) noted that long-term treatment with fluoxetine induced a significant increase in the contractile response to a higher concentration of noradrenaline. In contrast, it has been shown that paroxetine inhibited the contractile responses to noradrenaline, KCl and EFS on rat isolated vas deferens (Yaris et al., 2003). Similarly, Medina et al. (2000) reported that sertraline and fluoxetine reduced the adrenergic contractions of the human vas deferens. However, in this study noradrenaline-evoked contractions were not changed in either portion of the vas deferens obtained from rats treated long-term with fluoxetine or venlafaxine. This contradiction may in part be explained by the different species and experimental conditions used and by the duration of drug treatment.

In this study, ATP-induced contractile responses were decreased in both portions of the vas deferens obtained from fluoxetine- and venlafaxine-treated rats. Although the sympathetic co-transmitters ATP and noradrenaline both stimulate the contraction of isolated guinea-pig vas deferens, the natures of the respective contractions are markedly different (Han et al., 1987; Bean, 1992). Differences are most likely a reflection of the distinct signal transduction pathways activated by the two neurotransmitters. A possible explanation for impaired ATP contractions in both groups may include a postsynaptic damage. However, this possibility is unlikely as EFS-evoked contractions were not decreased in either group. ATP-evoked contraction appears to be related to an increase in ectonucleotidase activity. Since in this study, we did not perform use a non-hydrolyzable analogue of ATP, such as alpha, beta methylene ATP, we cannot draw any conclusion at the present time. The lack of effect of venlafaxine or fluoxetine treatment on noradrenaline-evoked contractions may be explained by purinergic receptors or post-receptor mechanisms being more sensitive to these treatments than noradrenergic receptors or post-receptor mechanisms.

In conclusion, this study demonstrates that chronic treatment with fluoxetine and venlafaxine affects vas deferens motility. Purinoceptors may, at least in part, be responsible for the impaired

**Figure 3** ATP concentration–responses curves in isolated epididymal segment of rat vas deferens smooth muscles. Each point is expressed as a percentage of the contraction induced by 80 mM KCl is given as the mean ± standard error of the mean (SEM). Number of rats in each group is shown in parentheses.

**Figure 4** ATP concentration–responses curves in isolated prostatic segment of rat vas deferens smooth muscles. Each point is expressed as a percentage of the contraction induced by 80 mM KCl is given as the mean ± standard error of the mean (SEM). Number of rats in each group is shown in parentheses.
motility in chronic treatment of venlafaxine and fluoxetine. Also, the present results might indicate the possible clinical implications of male reproductive function in patients undergoing SSRI or SNRI antidepressant medication.

References


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